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13. ABSTRACT *(Maximum 200 Words)*

Cancer is a multi-step process resulting from a number of genetic changes in cells. The role of tumor suppressor genes in breast carcinogenesis, especially at the early stage, remains largely unknown. We hypothesize that during the transformation of a breast epithelial cell, loss of function of several yet unidentified genes (tumor suppressors) results in either a partially or fully transformed phenotype. The aim of this study is to identify novel tumor suppressor genes involved in breast epithelial transformation using the gene-trapping technique. We used the polyA-trap retroviral vector pRET for infection into non-tumorigenic human mammary epithelial cells MCF10A. We screened for clones where functional genes have been "trapped" by selection for G418-resistance. We essentially established a gene-trapped library of MCF10A clones where expression of a single gene per clone is disrupted. We screened for transformed clones using the soft-agar cloning assay for anchorage independent growth. We isolated 25 transformed clones and identified the trapped genes in 5 clones by rapid amplification of cDNA ends (3'RACE). We identified 2 known genes and 2 novel genes as putative tumor suppressor genes. Further characterization of these genes will elucidate their role in the early transformation process in breast epithelial cells.

14. SUBJECT TERMS
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INTRODUCTION:

Cancer is a multi-step process resulting from a number of genetic changes in cells. With the exception of familial breast cancer cases where mutation in BRCA1 and BRCA2 are involved, the role of tumor suppressor genes in breast carcinogenesis, especially at the early stage, remains largely unknown. We hypothesize that during the transformation of a breast epithelial cell, loss of function of several yet unidentified genes (tumor suppressors) results in either a partially or fully transformed phenotype. The aim of this proposal is to identify novel tumor suppressor genes involved in breast epithelial transformation using the gene-trapping technique. Gene-trapping, a form of insertional mutagenesis, has been effectively used in identifying genes that are activated or inhibited in embryonic stem cells in response to different genotoxic agents or developmental signals. A DNA vector can be introduced into cells by transfection or infection and be randomly integrated into the genome in regions where functional genes are found. Insertion of the vector causes disruption of gene function. In this report, we describe how combination of gene-trapping and soft-agar cloning assay can be effectively used in identifying putative tumor suppressor genes that are involved in the early stages of breast transformation.

BODY:

1. Background

Breast cancer and tumor suppressor genes

Breast Cancer is the most common malignancy in women and the second leading cause of cancer-related death for women in the United States. More than 180,000 women are diagnosed with breast cancer each year [1]. The exact causes of breast cancer are not known. However, studies show that the risk of breast cancer increase as a woman gets older. There are both genetic and environmental components associated with breast cancer. Up to 10% of breast cancer in western countries is due to genetic predisposition. Although it is known that most hereditary and familial cases are due to mutations in the breast cancer gene1 (BRCA1), BRCA2 [2, 3], ataxia-telangiectasia (ATM) and the tumor suppressor gene p53 [4-6], the role of tumor suppressor genes associated with increased risk of breast cancer, especially at the early stage in its progression, still remain largely unknown. Thus, to identify novel tumor suppressor genes of human breast cancer, which especially played a role in the early stage of breast carcinogenesis, we need to study the effect of "loss of function" of a gene product in the cells, and then clone and characterize the putative tumor suppressor gene.

What is Gene trapping?

Since the sequence analysis of the human genome has already been completed, there is an increasingly urgent challenge to understand the fundamental function and interplay of genes that build and maintain an organism. Efficient strategies for functional studies will be required in the post-genomics era. Among the methods for functional studies of novel genes, gene trapping is straightforward and commonly used in mammalian organisms [7-13].

Gene trapping is a method of random insertional mutagenesis that uses a fragment of DNA coding for a reporter or selectable marker gene as a mutagen. Unlike chemical mutagens or X-ray, a mutation caused by DNA insertion tags the locus with a known sequence and allows rapid molecular characterization. This is an invaluable advantage in an animal with a large genome, small litters and

high maintenance costs [8, 14]. A DNA vector can be introduced into cells by transfection or infection and be randomly integrated into the genome in regions where functional genes are found. The sequence of the 'trapped' gene can be identified using techniques that are based on the polymerase chain reaction (PCR), and this can lead to the isolation of novel genes regardless of their level of expression in vivo [15].

A poly-A trap retrovirus vector, RET (removable exon trap), constructed by Ishida and Leder [15] uses a combination of a very strong splice acceptor, an effective polyadenylation signal and a promoterless green fluorescent protein cDNA that allows the expression pattern of the trapped gene to be monitored in living cells. An additional advantage of this vector is that the integrated proviruses can be removed from the genome of infected cells by excision using homologous recombination. Thus, it is possible to directly attribute the mutant phenotype to the vector integration by inducing phenotypic reversion following excision of the provirus.

2. Objective:

The specific aim of this proposal is to identify novel tumor suppressor genes involved in breast epithelial transformation using the gene-trapping technique.

3. Results:

(1) Establishment of a gene-trapped library of clones from human mammary epithelial cells (MCF10A)

Mounting evidence suggests that gene products may function differently depending on cell type, developmental stage, or species. Thus, to identify tumor suppressor gene(s) critical for the initiation of breast cancer, we need to study the effect of "loss-of-function" of a gene product in human breast epithelial cells. To analyze the effect of loss of every gene product in human mammary epithelial cells, we established a gene-trapped library of clones from MCF10A by using the poly-A trap retrovirus vectors (RET).

We generated virus containing the pRET vector by transfecting Phoenix amphotrophic retrovirus-producing mouse cell line (from Dr. Gary Nolan, Stanford University) with the pRET vector. Media containing virus was collected and the viral titer determined by infecting NIH3T3 cells. We used media with high titer virus to infect immortalized normal human mammary epithelial cells (MCF10A). Retroviral infectivity of mammalian cells allow for one retrovirus to infect a single cell then the cell becomes impermissive to further infection. The retroviral vector randomly integrates into the genome of the target cells (MCF10A). Inherent to the design of the vector is the requirement of the neomycin-resistance gene (contained in the vector) to acquire a polyadenylation signal via integration into a functional gene [15]. Thus, selection by G418 screens for clones that have the vector integrated into, and in effect, disrupting a functional gene. The vector also contains a promoterless green fluorescent protein (GFP) cDNA whose expression becomes regulated by the endogenous promoter of the disrupted gene (Fig.1).

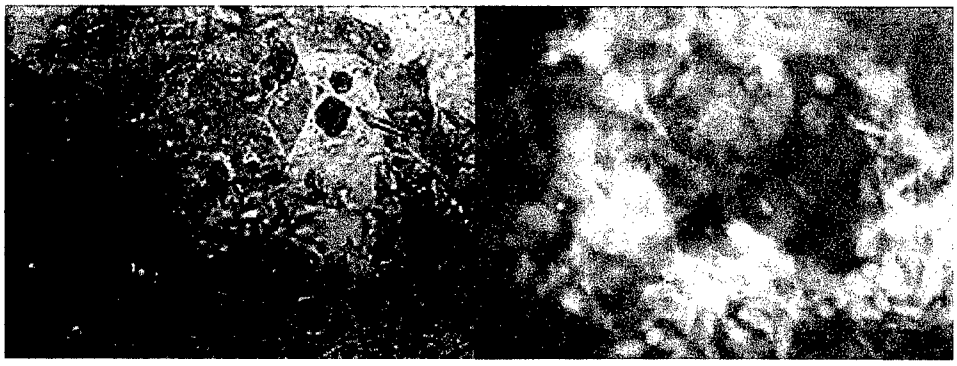

Figure 1: GFP expression in MCF10A infected cells
Left panel: MCF10A infected with pRET-1023 growing in tissue culture flask shown under normal light microscopy (40x magnification). Right panel: Same cells under fluorescence microscopy (40x magnification) showing GFP expression.

We screened G418-resistant and GFP-expressing cells and established essentially a gene-trapped library of human mammary epithelial cells. This library represented 3.0×10^5 independent infected clones in which one allele of a functional gene was disrupted by the retrovirus gene searching vector. Expression from the other allele can be inhibited by using an inducible antisense vector [16] or small RNA inhibitors (RNAi) [17]. The library is an asset to scientific community since it is useful for screening specific genes if "loss-of-function" results in detectable phenotypic or biochemical changes.

(2) Cloning potential human breast tumor suppressor genes from the gene-trapped library of MCF10A cells

As hypothesized in our proposal, the loss of a tumor suppressor gene, important for initiation of breast cancer development, can result in the transformed phenotypic changes in human breast epithelial cells. These phenotypic changes involve the loss of anchorage-dependent growth. To assay for this specific phenotypic change, we performed soft agar cloning assay. We identified cells that have acquired anchorage independent growth and analyzed expression of GFP (Fig. 2).

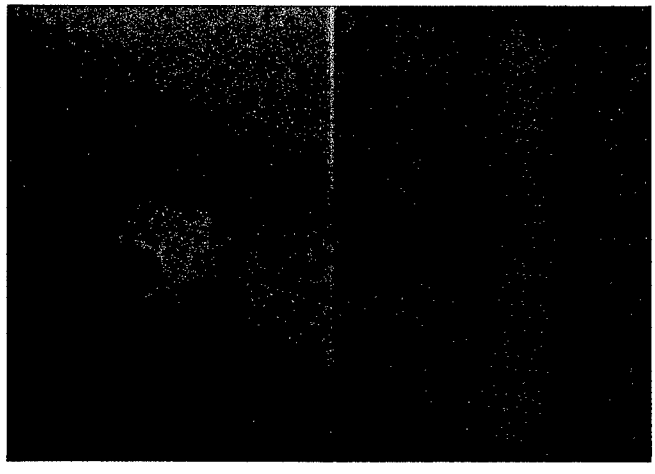

Figure 2. Expression of GFP in pRET- infected MCF10A cells growing in soft agar. Left panel: Fluorescence microscopy (GFP expression); Right panel: Light microscopy (colony morphology) 160x magnification

From the soft agar cloning assay, more than 100 positive colonies (>0.2 mm diameter) grew in soft agar from the gene-trapped library of clones. The parental line MCF10A did not form any colonies. So far we have isolated 25 of the transformed colonies (Fig.3) and expanded them to further analyze their growth characteristics (MCF10A-1011: 10 colonies; MCF10A-1023: 15 colonies).

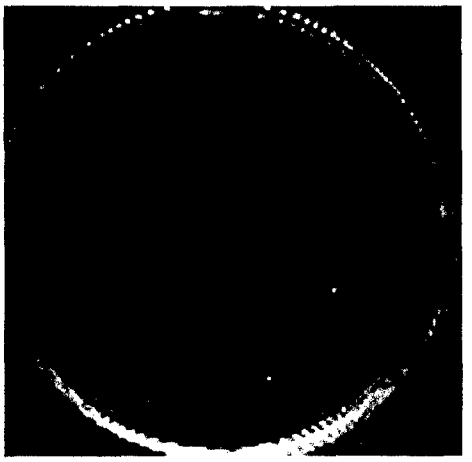

Figure 3: Transformed MCF10A colonies growing in soft agar. White spots shown are colonies growing in soft agar (16x magnification). These colonies were isolated and expanded for further analysis of their transformed phenotype.

To clone potential human breast tumor suppressor genes from the cells that have acquired anchorage-independent growth, we used 3'RACE (Rapid Amplification of cDNA ends) and sequencing. 3'RACE takes advantage of the natural poly (A) tail found in mRNA as a generic priming site for PCR. In this procedure, mRNAs are converted into cDNA using reverse transcriptase (RT) and an oligo-dT adapter primer. Specific cDNA is then amplified by PCR using a gene-specific primer that anneals to a region of known exon sequences and an adapter primer that targets the poly (A) tail region. This permits the capture of unknown 3'mRNA sequences that lie between the exon and the poly (A) tail. The purified PCR fragment(s) were cloned into a sequencing pCR4-TOPO vector using TOPO TA Cloning kit for sequencing from Invitrogen. We used M13 universal primers for sequencing the PCR fragment (Davis Sequencing, CA) (Fig.4)

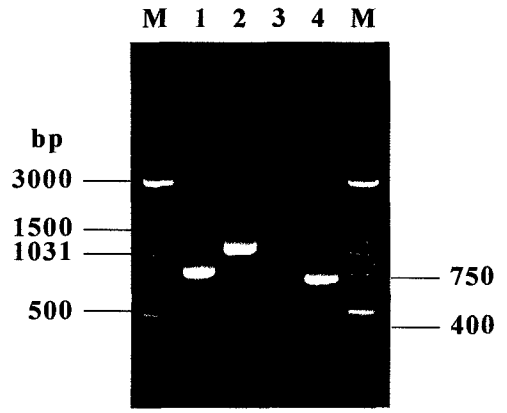

Figure 4: Rapid Amplification of cDNA ends (3'RACE) to identify trapped genes. PCR products from 3'RACE were electrophoresed in 1.5% agarose gel and fragments were isolated for further cloning and sequencing. M= molecular weight marker in base pair (bp); Lane 1: ST1, Lane 2: ST4, Lane 3: ST6, Lane 4: positive control (TOPO)

Sequences obtained were used for database search using BLASTN and BLASTX from the GenBank. We were able to obtain both cDNA and genomic sequences from the search. The 5 sequences of the trapped genes have been found to match with sequences from the GenBank from

different chromosomes in human genomic DNA. The results of the BLAST search are shown in Table 1.

Table 1:

Name of Clone	Identities of Sequence	Locus in GenBank	Chromosome	Trapped Gene	Length (bp)
1023-ST4	98%	AL359555.9	20	Putative novel gene (bK2308N23.2)	678
	98%	NT_011362.5	20		
1011-ST1	99%	XM_048940	7	Caveolin-1	641
	99%	4572326	7	Caveolin-1 exon-3	
1011-ST6	97%	6599139	?	human testis mRNA (DKFZP434J186)	668
1011-ST3	92%	NT_007927.5	7	Caveolin-1	
1023-ST14	97%	NT _004441.5	1	SPRRR1B (cornifin)	710

Identification of caveolin-1 (Cav-1) as one of the trapped genes that produced a transformed phenotype serves as a positive control for our system. Several studies have suggested that Cav-1, the main structural protein of caveolae, also functions as a tumor suppressor [18] [19]. A recent report indicates that the Cav-1 gene is mutated in up to 16% of human breast cancer samples [20]. Antisense down-mediated down-regulation of Cav-1 in NIH3T3 fibroblasts led to a hyperactivation of the MAP kinase pathway and anchorage–independent growth [21]. Down-regulation of Cav-1 has been reported in several types of tumors including breast, colon, ovarian and sarcomas [22-31]. Taken together, these studies support the proposed function of Cav-1 as a tumor suppressor whose reduction/deletion in cells will provide growth advantages and expedite tumorigenesis. Our findings further support the role of Cav-1 as a tumor suppressor in breast cancer.

The sequence for ST4 matched with a sequence from human chromosome 20 that codes for a novel putative gene. The sequence of the cDNA clone indicates an open reading frame of 269bp, potentially coding for a protein of only 89 amino acids. We have obtained the full-length cDNA clone and plan to express the encoded protein in bacteria to further characterize the encoded product by this putative gene. We are currently using Southern and Northern blot analysis to study the genomic structure and expression of this gene. We will be performing similar analysis for the other novel gene, ST6, to elucidate its function as putative tumor suppressor in breast transformation.

Cornifin (SPRRR1B) is the latest gene we have identified from one of the transformed clones. Cornifins/small proline-rich proteins (SPRRs) belong to a family of proline-rich proteins that function as cornified envelope precursors [32]. Expression of cornifin is specific for squamous differentiation although senescing keratinocytes have also been shown to express genes such as

cornifin [33] [34] [35]. These changes were similar to those observed in keratinocytes induced to differentiate with phorbol ester or by confluence. Cornifin were similarly altered in senescing human mammary epithelial cells. A recent study showed decreased expression of cornifin in cervical intraepithelial neoplasia [36]). With its current role as a differentiation marker, further study of its putative role as a tumor suppressor may open up more possibilities and better understanding of its function in epithelial cell transformation. We plan to further analyze this function of cornifin in breast cancer.

(3) Characterization of the Transformed MCF10A clones

A. Measurement of cell proliferation

To characterize the growth of the transformed MCF10A breast epithelial clones in culture, we analyzed the cell proliferation rate by using a colorimetric method based on the cellular conversion of a tetrazolium salt into a formazan product (Cell Proliferation Assay, Promega, WI). The assay was performed every 24 hours for at 4 days to generate cell growth curves. The growth of the transformed clones (ST-1, ST4, ST8) was faster than parental MCF10A cells (Fig.5). ST8 is one of the transformed clones we have isolated but have not yet identified the trapped gene. These results suggest that disruption of the trapped genes in each of the transformed clones may provide a growth advantage for the cells in culture.

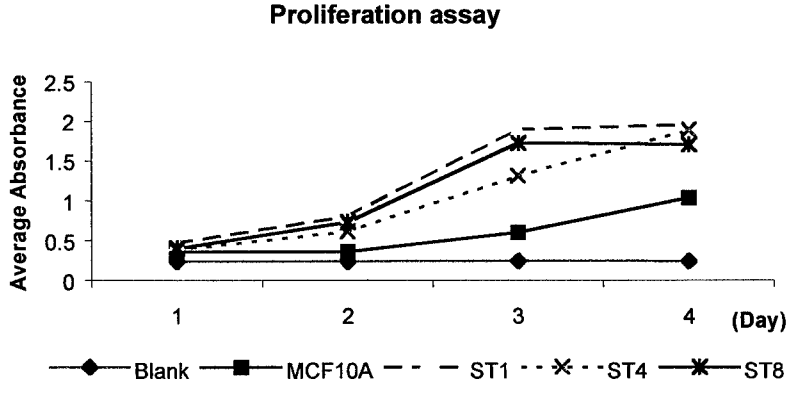

Figure 5: Growth of MCF10A parental and transformed clones. Cells were plated in 96 well plates in complete media and cell proliferation rates were measured every 24 hours. Average absorbances of 4 wells/clone are shown. Blank = media alone; ST8= transformed clone with yet unidentified trapped gene

B. Caveolin-1 expression in the trapped genes clones

To determine that the integration of the pRET vector disrupted expression of cellular genes in MCF10A, we analyzed caveolin-1 mRNA and protein expression in the 1011-ST1 cell clone, where caveolin-1 (Cav-1) gene had been trapped. We used Cav-1 cDNA fragment as a probe for Northern blot analysis (Figure 6) and anti-Cav-1 antibody (N-20) for western blot analysis (Figure 7). Results show that both mRNA and protein expression of caveolin-1 in parental MCF10A is about 2-fold higher compared to clone ST1 (Cav-1 trapped clone). This is consistent with our predicted decreased expression when only one allele is disrupted and one functional allele is present. It essentially creates

haploinsufficiency which, for Cav-1 in our assay, is enough to cause breast cell transformation into anchorage-independent phenotype.

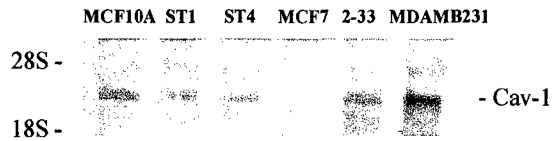

Figure 6: Northern blot analysis of Caveolin-1 expression. Five μg of total RNA from several cell lines were analyzed by Northern blot using Cav-1 cDNA as probe. MCF7 breast adenocarcinoma cell line serves as negative control for Cav-1 expression while 2-33 and MDA-MB231 are high expressors of Cav-1.

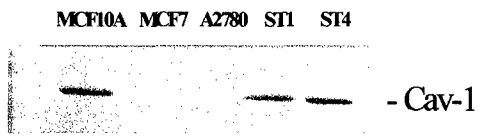

Figure 7: Western blot analysis of Caveolin-1 expression. Equal amounts of total cellular extracts from various cell lines were analyzed by western blot using rabbit anti-Cav-1 antibody (N20). MCF7(breast cancer cell line) and A2780 (ovarian cancer cell line) serve as negative control for Cav-1 expression

C. Excision of integrated provirus for reverse transformation

To confirm that the resulting phenotype was due to provirus insertion, we transfected an excision vector (pCAGGS-Cre-T, courtesy of Dr. Philip Leder) into the MCF10A transformed cells (ST1 and ST4). PCAGGS-CreT encodes for an enzyme can cut the inserted retroviral vector out[16]. Transfected cells were selected using gancyclovir. We analyzed GFP expression by immunofluorescence to determine whether the inserted retroviral vector has been excised (Figure 8). As shown in fig.8, GFP expression is lost upon transfection of the excision vector. Gancyclovir-resistant clones are growing slowly in culture. We will proceed with soft agar cloning assay when we have more cells to determine whether these clones have lost their anchorage independent growth. This will ensure that the transformed phenotype resulted from retroviral gene trapping, not from spontaneous processes.

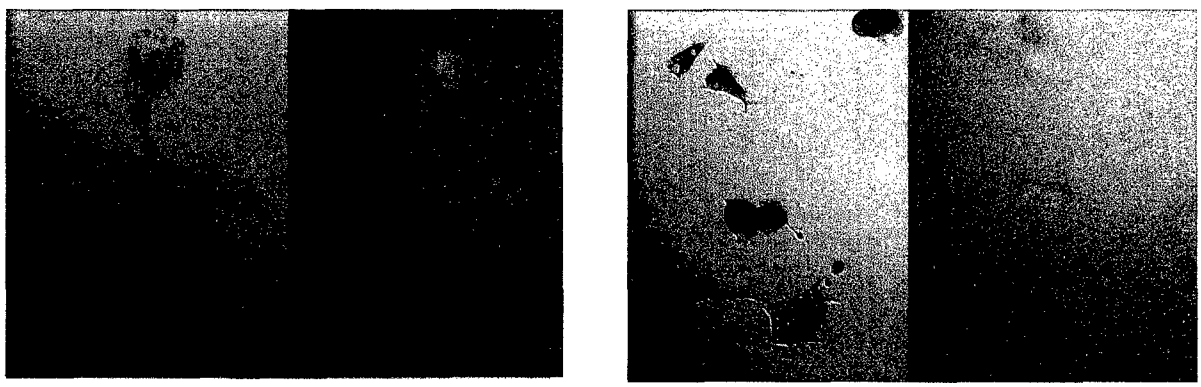

Figure 8: Loss of GFP expression in pCAGGS-Cre-T transfected in MCF10A-ST1. Left panel: parental ST1 clone showing GFP expression by immunofluorescence. Right panel: ST1 clone transfected with pCAGGS-Cre-T showing no detectable GFP expression by immunofluorescence (40x magnification).

D. Microarray analysis of differential gene expression in transformed clones

Microarray technology allows researchers to measure the expression levels of thousands of genes simultaneously over different time points, different experimental conditions or different tissue. In order to elucidate the mechanism of tumor suppression by the identified trapped genes, we started by analyzing gene expression profiles of the transformed clones compared to the parental MCF10A. We analyzed 281 of human cancer related genes using the NEN Microarray TSA and directly labeling system for 1011-ST1 and 1023-ST4 clones. We found that in clone 1011-ST1 the expression of 16 genes was up-regulated (2-fold or higher) while 36 genes were down-regulated. In clone 1023-ST4, the expression of 4 genes was up-regulated (2-fold or higher)and 51 genes were down-regulated (Table 2). Notably, a number of putative tumor suppressor genes were also found to be down-regulated in both clones (ST1 and ST4) examined. We are currently confirming the results of the microarray by Northern blot analysis of specific genes that may play a role in the acquisition of anchorage-independent growth and transformation of breast epithelial cells. Results from these analyses will direct our future effort in understanding the function of the putative novel genes, as well as other functions of the known genes such as caveolin-1 and cornifin.

Table 2:

Genes down-regulated in ST4 clone

bcl-1.
Lymphocyte specific interferon regulatory factor/interferon regulatory factor 4 (LSIRF/IRF4).
Homozygous deletion target in pancreatic carcinoma (DPC4).
Ubiquitously expressed transcript (UXT).
Helix-loop-helix zipper protein (max).
MET proto-oncogene.
c-yes-1 mRNA.
Cdc7 (CDC7).
Evi-1, Evi-1 protein
Putative tumor suppressor (LUCA15).
Factor KBF1.
XMP.
mRNA for Net transcription factor.
E2k, alpha-ketoglutarate dehydrogenase complex dihydrolipoyl succinyltransferase.
Putative tumor suppressor ST13 (ST13).
RNA for c-fes.
Putative tumor suppressor protein (101F6).
mRNA for FOP (FGFR1 oncogene partner).
tra1 mRNA forhomologue of murine tumor rejection antigen gp96.
fra-1 mRNA.
Adenocarcinoma-associated antigen (KSA).
Bcl2, p53 binding protein Bbp/53BP2 (BBP/53BP2).
Prot-oncogene (BMI-1).
FIP2 alternatively translated.
mRNA for CRK-II.
Int-6.
Tumor susceptibility protein (TSG101).
GTP-binding protein (RALB).
Candidate tumor suppressor gene 21 protein.
BB1, malignant cell expression-enhanced gene/tumor progression-enhanced gene.
cDNA for RFG.
H4(D10S170), putative cytoskeletal protein.
C-abl gene.
Transcriptional repressor (CTCF).
Putative tumor suppressor protein (RDA32).
mRNA for tre oncogene (clone 210).
Ser/Thr protein kinase (A-RAF-1) gene
mRNA for raf oncogene.

mRNA for ch-TOG protein.
9G8 splicing factor.
Tumor suppressing STF cDNA 4 (TSSC4).
mRNA for NF-kB subunit.
Kruppel related gene, exon X, clone pGLI2RR.
Beclin 1 (BECN1).
Cytosolic aspartate aminotransferase.
mRNA for APO-1 cell surface antigen.

Fumarase precursor (FH) mRNA, nuclear gene encoding mitochondrial protein.

DNA-binding protein (GLI3).
Succinate dehydrogenase iron-protein subunit (sdhB) gene.

Putative lung tumor suppressor (DAL1).

Genes up-regulated in ST4

mRNA for MAT8 protein.
Epithelium-restricted Ets protein ESX.
rap2 mRNA for ras-related protein.

B-cell lymphoma 3-encoded protein (bcl-3).

Genes down-regulated in ST1

Tumor suppressor gene, P16/MTS1/CDKN2, cell cycle negative regulator beta form.
PMI1 mRNA for phosphomannose isomerase.
mRNA for transmembrane epithelial tumour mucin antigen.
mRNA for RCK.
Kruppel related gene, exon X, clone pGLI2RR.
Tyrosine kinase receptor (axl).
FIP2 alternatively translated.
mRNA for precursor of epidermal growth factor receptor.
mRNA for carcinoembryonic antigen family member 2, CGM2.
mRNA for c-fms proto-oncogene.
MAFB/Kreisler basic region/leucine zipper transcription factor (MAFB).
B94 protein.
DNA-binding protein (HRC1).
Transactivator (jun-B) gene
CUL-2 (cul-2).
sarcospan-2 (SPN2).
Putative tumor suppressor ST13 (ST13).
Epidermal growth factor receptor (HER3).
(clone lambda-Ki-4) c-Ki-ras1 pseudogene; region homologous to c-Ki-ras2 gene

Rel proto-oncogene mRNA.
Lung cancer antigen NY-LU-12 variant A.
PBX3 mRNA.
Tyrosine kinase-type receptor (HER2).
(E2F-1) pRB-binding protein.
mRNA for NF-kB subunit.
mRNA for TL132.
Protein tyrosine phosphatase (TEP1).
Leucine zipper protein Fip3p.
CDK4-inhibitor (p16-INK4).
C-abl gene.
mRNA for transcription factor, Maf.
Erythroblastosis virus oncogene homolog 2 (ets-2).
Low molecular mass GTP-binding protein (ral) mRNA
Helix-loop-helix zipper protein (max).
Cdc7 (CDC7).

Genes up-regulated in ST1

Chaperonin protein (Tcp20) gene complete cds.
Fumarase precursor (FH) mRNA, nuclear gene encoding mitochondrial protein.
Succinate dehydrogenase iron-protein subunit (sdhB) gene.
Lyn mRNA encoding a tyrosine kinase.

mRNA for beta-actin.
mRNA for lactate dehydrogenase-A (LDH-A, EC 1.1.1.27).
mRNA encoding phosphoglycerate kinase.
TROP-2 gene.
Phosphoglycerate mutase (PGAM-B).
Cytosolic aspartate aminotransferase.
mRNA for HMG-1.
tra1 mRNA forhomologue of murine tumor rejection antigen gp96.
Tumorous imaginal discs protein Tid56 homolog (TID1).
Tumor antigen (L6).
mRNA for tumor-associated 120 kDa nuclear protein p120(carboxyl terminus).

Alpha-tubulin.

Tumor necrosis factor alpha inducible protein A20.
Secreted frizzled related protein.

KEY RESEARCH ACCOMPLISHMENTS:

- Created a gene-trapped library of human breast epithelial cell line MCF10A clones
- Identified 2 novel putative tumor suppressor genes (ST4 and ST-6) involved in breast epithelial transformation
- Identified 2 known genes (caveolin-1 and cornifin) that may function as tumor suppressors in breast epithelial transformation
- Showed that decreased expression of Cav-1 is sufficient for phenotypic transformation of MCF10A cells

REPORTABLE OUTCOMES:

Funding applied for based on work supported by this award:

1) Breast Cancer Research Program 2001
Post-doctoral Fellowship Award (submitted June 2001)
Title: Identification and Characterization of Putative Tumor Suppressor Genes Involved in Early Breast Carcinogenesis
Principal Investigator: Wei Zou, Ph.D.
Agency: Dept. of Defense

2) Breast Cancer Research Program 2001
Pre-doctoral Traineeship Award (submitted June 2001)
Title: Identification of novel genes affected by gamma irradiation using a gene trapped library of human mammary epithelial cells
Principal Investigator: Jennifer Malone, Ph.D. Candidate
Agency: Dept. of Defense

Personnel supported by this grant:
Dr. Wei Zou (postdoctoral fellow)
Kelle Phelps (laboratory technician)

CONCLUSIONS:

Gene-trapping technique is an effective and powerful technique that we have shown to work successfully in human mammary epithelial cells. Using a combination of gene-trapping technique and screening for anchorage-independent growth for transformation, we have identified 2 novel putative tumor suppressor genes (ST4 and ST-6) in early breast transformation. We have also identified 2 known genes, caveolin-1 and cornifin, that may function as tumor suppressors in addition to their known physiologic roles in cellular structure and differentiation. Our system allows us to directly isolate clones for characterization of the effects of targeted disruption of specific genes both in vitro and in vivo. Further analysis of the transformed clones and the trapped genes will lead to a better understanding of the mechanisms of action of the product of these genes. We expect to identify more genes from the gene-trapped library whose functions are important in the maintenance of the normal breast cell phenotype.

The results from this study emphasize the need for more research on the function of gene products. With the availability of the sequence of the whole human genome, the next step is identifying the functions of these genes and the consequences of their loss of function or deregulation of expression. Identification of tumor suppressor genes that are responsible for the initiation of breast cancer will be useful in finding biological markers for early detection of the disease or lead to the development of therapeutic reagents to prevent breast cancer progression.

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DEPARTMENT OF THE ARMY
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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 28 Aug 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

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2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLIS M. RINEHART
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